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RESEARCH

Genomic patterns of native palms from the Leeward Antilles confirm single-island endemism and guide conservation priorities

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Abstract

Systematic study of the palm (Arecaceae) flora from the Leeward Antilles marked both *Sabal antillensis* and *Sabal lougheediana* as endemic to Curaçao and Bonaire respectively. Although both species are emergent, obvious and charismatic elements of the Antillean flora, they were only described within the last decade. *Sabal lougheediana* is in demographic crisis as a result of constant ungulate pressure prohibiting seedling establishment, while *S. antillensis* is thriving as a result of careful management. Given the surprisingly recent discovery of these two species, and the severity of the conservation concern, genomic data can help inform whether these taxa are in fact well-circumscribed species, and help to guide further conservation actions. To investigate genomic diversity, we employed RADSeq data from samples throughout the range of both species. Results show significant genetic distance and fixation between the two taxa as currently circumscribed, as well as reduced genetic diversity and increased inbreeding in both species. Multivariate analysis of genetic distance and Bayesian clustering analysis both show clear and significant separation of the two taxa. Parallel assay of ex situ collections informs how future development of germplasm reserves can help support conservation of both species. We provide recommendations to help conserve these two unique and distinct species.

Keywords Arecaceae · Herbivore pressure · *Sabal* · Taxonomy

Abstract

Systematisch onderzoek van de palmflora (Arecaceae) van de Benedenwindse Antillen markeerde zowel *Sabal antillensis* als *Sabal lougheediana* als endemisch voor respectievelijk Curaçao en Bonaire. Hoewel beide soorten aspect-bepalende, typische en charismatische elementen van de Antilliaanse flora zijn, zijn ze pas in de afgelopen tien jaar beschreven. *Sabal lougheediana* verkeert in een demografische crisis als gevolg van de voortdurende druk van grazers die de vestiging van zaailingen verhindert, terwijl *S. antillensis* gedijt als resultaat van zorgvuldig beheer. Gezien de verrassend recente ontdekking van deze twee soorten, en de ernst van de bezorgdheid over het behoud, kunnen genomische gegevens helpen bepalen of deze taxa in feite goed omschreven soorten zijn, en helpen om verdere natuurbehoudsmaatregelen op te zetten. Om de genomische diversiteit te onderzoeken, hebben we RADSeq-gegevens gebruikt van monsters uit het hele

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verspreidingsgebied van beide soorten. De resultaten laten een aanzienlijke genetische afstand en fixatie zien tussen de twee taxa zoals momenteel omschreven, evenals een verminderde genetische diversiteit en een toegenomen inteelt bij beide soorten. Multivariate analyse van genetische afstand en Bayesiaanse clusteranalyse laten beide een duidelijke en significante scheiding tussen de twee taxa zien. Parallelle tests van ex situ collecties geven aan hoe de toekomstige ontwikkeling van kiemplasmareserves het behoud van beide soorten kan helpen ondersteunen. We geven aanbevelingen om deze twee unieke en verschillende soorten te helpen behouden.

Trefwoorden Arecaceae · begrazingsdruk · *Sabal* · taxonomie

Apstrakto

Estudio sistemátiko di flora di palma (Arecaceae) di Islanan Abou a marka tantu *Sabal antillensis* komo *Sabal lougheediana* komo endémiko na Kòrsou i Boneiru respektivamente. Aunke tur dos espesie ta elementonan emergente, opvio i karismátiko di flora antiano, ta te den e último dékada a deskribí nan. *Sabal lougheediana* ta den krísis demográfiko komo resultado di preshon konstante di ungulado strobando stablesimentu di mata chikitu, miéntras *S. antillensis* ta prosperá komo resultado di maneho kuidadoso. Mirando e deskubrimentu resien sorprendente di e dos espesienan akí, i e severidat di e problema di konservashon, datonan genómiko por yuda informá si e táksònnan ei realmente ta espesienan bon definí, i ta yuda guia akshon di konservashon den futuro. Pa investigá diversidat genómiko, nos ta hasi uso di datonan di RADSeq for di muestranan di henter e área di distribushon di ámbos espesie. Resultadonan ta mustra distansia genétiko i fihamentu signifikante entre e dos táksònnan manera ta deskribí nan aktualmente, komo tambe diversidat genétiko redusí i oumento di endogamia den tur dos espesie. Tantu e análisis multivariante di distansia genétiko komo análisis di agrupashon bayesiano ta mustra separashon kla i signifikante di e dos táksònnan. Ensayonan paralelo di kolekshonnan ex situ ta informá kon futuro desaroyo di reserva di germoplasma por yuda sostené konservashon di tur dos espesie. Nos ta ofresé rekomendashon p.a. yuda konservá e dos espesienan úniko i distinto akí.

Palabranan Klave Arecaceae · preshon hèrbívoro · *Sabal* · taksonomia

Introduction

The Leeward Antillean flora, in the islands running east to west along the southern edge of the Caribbean, includes one confirmed native palm genus, *Sabal* Adans., with two species present, *Sabal antillensis* M.P.Griff. and *Sabal lougheediana* M.P.Griff. & Coolen (Fig. 1). *Copernicia tectorum* (Kunth) Mart. also occurs on Curaçao but is variously considered either native or introduced (Van Buurt 2009, Van Proosdij 2012). *Sabal* species on these islands were long known (Arnoldo 1954, 1964, Winkelman 1979, Van Proosdij 2001, 2012) but not given direct taxonomic attention until recently, only being described within the last decade (Griffith et al. 2017, 2019a). The two species show geographic and morphological separation and cohesiveness; *S. antillensis*, the Curaçao Palm, is only found on the slopes of Christoffelberg and hills to the west of that summit, and shows pronounced pachycauly and pendescent leaflets, while *S. lougheediana*, the Bonaire Palm, is restricted to a very small area of limestone terrace in southern Bonaire, does not show pronounced pachycauly, and holds leaflets erect; further differences, including habitat, habit, ecology, and anatomy, are detailed in Griffith et al. (2019a). Each species is limited to a small geographic range (Winkelman 1979; De Freitas et al. 2019). Thorough survey of each species in 1978 and 2018 found a robust, healthy demography for *Sabal antillensis*, with abundant seedlings and subadults (De Freitas et al. 2019), but signs of sustained herbivore pressure in *Sabal lougheediana*, leading to a mature stand with very few subadults and seedlings to replace senescing palms (cf. Maunder et al. 2002, Klimova et al. 2021). The numbers of mature *S. antillensis* increased over that 40-year period to well over 1,200 adult palms, whereas the *S. lougheediana* census fell precipitously from 31 to 25 adult palms (De Freitas et al. 2019). In addition, palms on Bonaire have been overexploited for thatch in earlier times (Winkelman 1979), although this cultural use is no longer an immediate concern (Griffith et al. 2019a).

Given the geographic proximity of these two species, which excludes any other *Sabal* in these islands, a close relationship has been assumed (Griffith et al. 2019a). The genus includes 17 species from the southern United States through Northern South America. The most geographically proximal *Sabal* species to the Leeward Antilles is *Sabal mauritiiformis* (H.Karst.) Griseb. & H.Wendl., found in Venezuela and ranging north and west through Central America and Southern Mexico (Henderson et al. 1995). Beyond *S. mauritiiformis*, the nearest congener is *S. causiarum* (O.F.Cook) Becc., found in the Greater

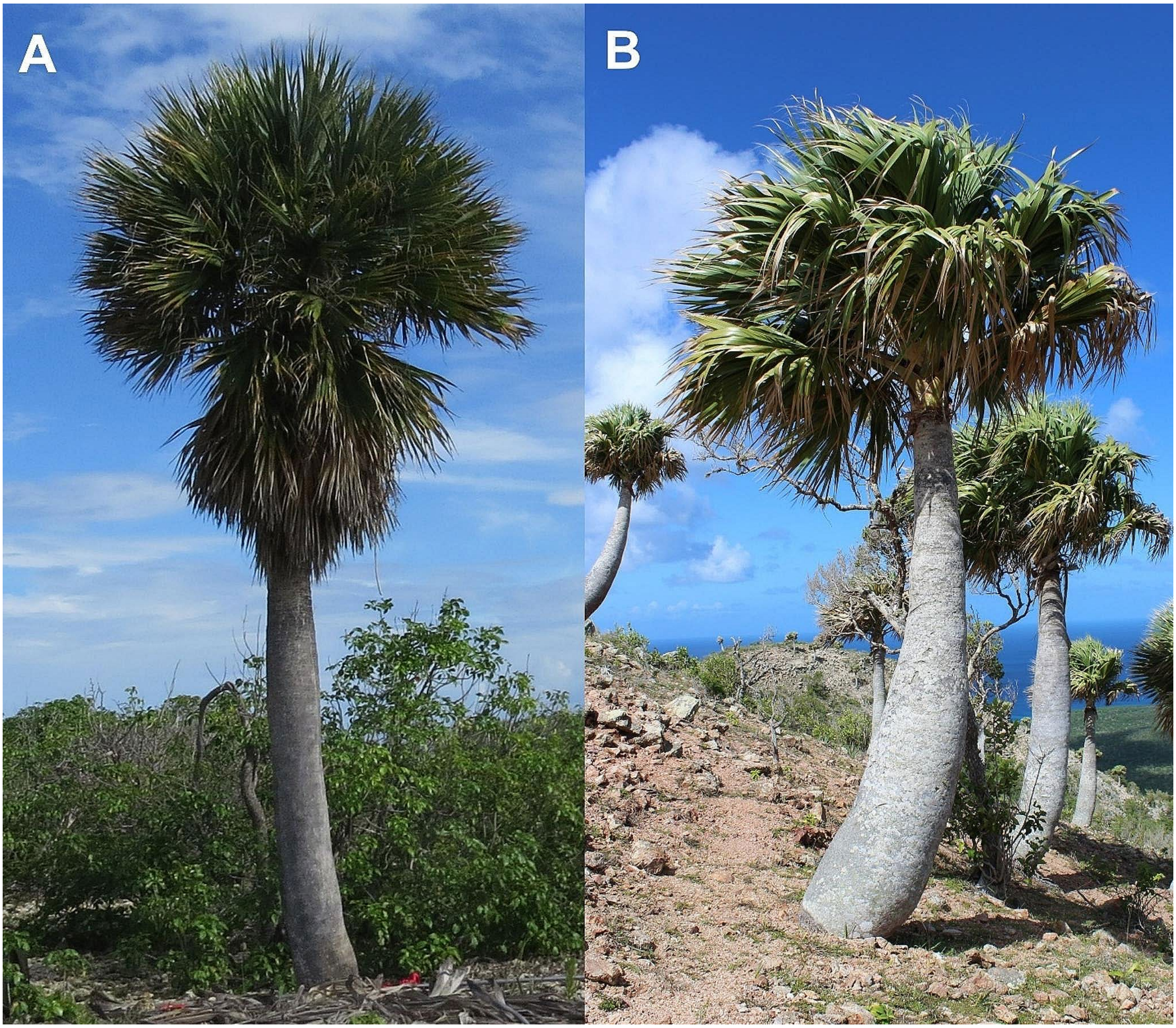

Fig. 1 **(A)***Sabal lougheediana* (Kabana di Boneiru; Bonaire Palm) growing on a limestone terrace in Southeastern Bonaire. **(B)***Sabal antillensis* (Kabana di Korsou; Curaçao Palm), growing on a chert hill in Northwestern Curaçao. Both species are dominant and emergent members of their respective florae and vegetation. Figure adapted with permission from Griffith et al. (2019a)

Antilles (Henderson et al. 1995). *Sabal palmetto* (Walter) Lodd. ex Schult. & Schult.f. is also used in civic landscaping on Curaçao, and could pose an invasive risk, or a vector for pests.

Conservation concern for the Leeward Antillean palm species has been put forward (De Freitas et al. 2019), even prior to these plants being determined to species level; Winkelman et al. (1979) and De Freitas et al. (2005) noted that the "*Sabal* spec." on each island needed protection. The major herbivores are feral goats and donkeys, which consume entire seedlings and young palms. De Freitas et al. (2019) demonstrated the very positive effects of removing and excluding these herbivores from the palms' habitat in Curaçao, and called for similar action in Bonaire. As a response, an emergency exclusion fence on Bonaire was completed in 2022 (Fig. 2), and this management effort is currently being expanded to encompass the native range of the Bonaire Palm. These efforts are now part of a broader official framework for resource conservation, cultural conservation and sustainable development for southern Bonaire (Engel et al. 2022). Additionally, ex situ living collections were developed to complement these in situ protections, to act as a germplasm reserve in case of pathogens, saltwater intrusion (ibid.), or other factors causing catastrophic losses.

Fig. 2 Exclusion fence on Bonaire; outside of fence on left, inside on right. Overgrazing by introduced ungulates has limited the reproduction and recruitment of *Sabal lougheediana*. With the fence completed in 2022, this photo shows vegetation recovery underway. Survey in June 2023 located abundant one- and two-year seedlings within the exclosure

Related to these conservation actions are two questions. First, are these two species genomically distinct? Conservation investment in *Sabal lougheediana* in particular was greatly facilitated after its description as a unique and separate species, distinct from *S. antillensis*. Although we take the position that all native palm populations are worth conserving, our experience shows that confirmation or refutation of species status has bearing on the degree of effort put forward for protecting plant resources (cf. Morrison, 2009). While taxonomic descriptions are almost always morphologically-based (see examples in Article 38, International Code of Botanical Nomenclature; Turland et al. 2018), molecular data can confirm these descriptive classifications (Flores-Olvera et al. 2016). The fact that these two charismatic megaflora (cf. Crane 2015) species evaded formal description until so recently prompts questions about synonomy that can be addressed through molecular means. Discussion with local and international stakeholders highlighted a desire to evaluate these species boundaries through DNA data. So, here we test the hypothesis that *Sabal antillensis* and *S. lougheediana* are genomically distinct, as this has bearing on the degree of resources mobilized for their protection.

Second, are ex situ conservation efforts adequately representing the genomic diversity of each species? A revolution in rethinking and assaying botanic garden living collections for conservation genetic value is underway (e.g. Cibrian et al. 2013, Hoban et al. 2020), and genomic techniques can inform how well ex situ palm collections can carry forward the limited genetic diversity found in small populations (Diaz-Martin et al. 2023). Our study therefore aims to also test the hypothesis that ex situ collections of each species adequately represent in situ diversity. To answer both of these questions, we have gathered and genotyped in situ and ex situ population samples of the native palms from Bonaire and Curaçao.

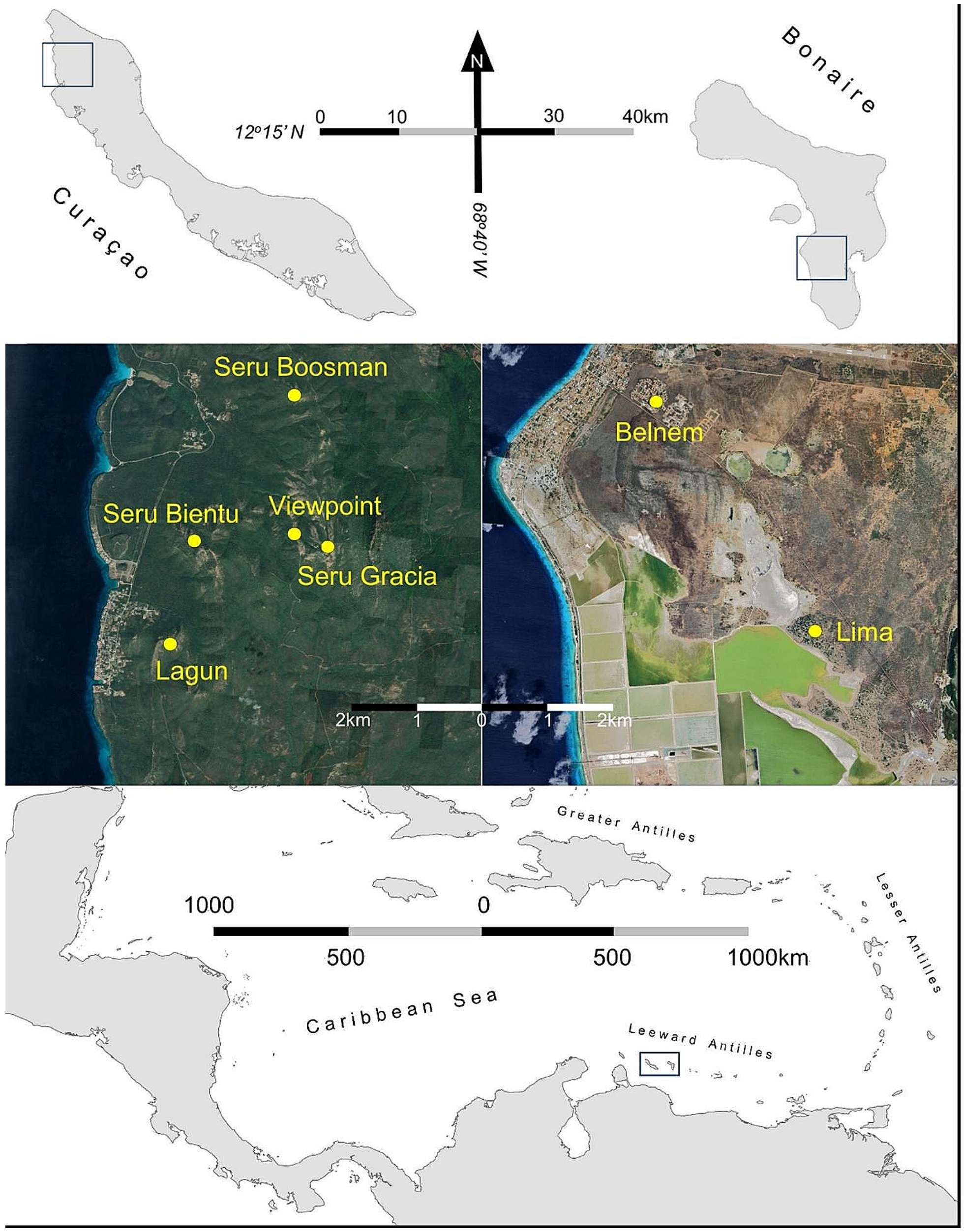

Fig. 3 Location of sampling sites on Curaçao (left) and Bonaire (right). The Belnem site was known to host *Sabal lougheediana* in past decades (Winkelman 1979) but the habitat is converted to residential development, and only a single wild palm is extant there. Lagun = two adjacent hilltops, Seru Pasku and Seru Para Mira

Materials and methods

Sampling

In situ samples were collected in September 2021 (Bonaire) and January 2022 (Curaçao). Plants were collected from throughout the geographic range of both species (De Freitas et al. 2019), and involved removing up to 5 leaflets per adult plant, cutting these into short sections, and drying and storing at room temperature on silica gel. We selected plants for sampling based on geography, seeking to sample from all edges of the range as well as interior points (see maps on Fig. 3). Based on sampling guidelines from Nazareno et al. (2017), 44 in situ plants from 5 sites throughout the range of *S. antillensis* on Curaçao were used in the study, along with 18 in situ plants of *S. lougheediana* from two sites on Bonaire (Fig. 3); the main population of *S. lougheediana* occurs at Lima, and a single relict palm remains at Belnem. Seven ex situ samples came from plants growing at Montgomery Botanical Center derived from seeds collected on each island in 2017 and 2018. Each of the seven ex situ samples used in the study came from a separate maternal line (Griffith et al. 2020). Table 1 provides an overview of the sampling structure, and Appendix 1 provides details on the plants used in the study.

Table 1 Sampling structure for genotyping of Leeward Antillean Palms

Taxon	Source	Cohort	N plants
Sabal lougheediana	Bonaire		
M.P.Griff	Belnem	In Situ	1
& Coolen	Lima	In Situ	17
	Montgomery	Ex Situ	4
Sabal antillensis	Curaçao		
M.P.Griff.	Lagun *	In Situ	3
	Seru Bientu	In Situ	14
	Seru Boosman	In Situ	4
	Seru Gracia	In Situ	14
	Viewpoint	In Situ	9
	Montgomery	Ex Situ	3
		Total	**69**

* Two adjacent hilltops above Lagun, Seru Pasku and Seru Para Mira

DNA extraction, library preparation and sequencing

DNA extraction, library preparation and sequencing were carried out by FLORAGENEX, INC (Oregon, USA). In summary DNA was extracted from fresh silica dried leaf material, quantified via a Qubit Quant iT dsDNA HS Assay system (Invitrogen), and then normalized to 500ng of purified DNA for restriction site associated DNA sequencing (RADseq sensu Flanagan and Jones 2018) using the *sbfI* restriction enzyme and then sequenced using the Illumina HiSeq 4000 genomic sequencer for 150 single end run/low density scan.

Assembly of RADseq data

First, Illumina reads were assessed for quality using FastQC 0.12.1 (Andrews et al. 2014). Quality checking, filtering and de novo assembly of the single-end reads was performed using ipyrad 0.9.87 (Eaton and Overcast 2020), in ipyrad all parameters were set to default unless stated otherwise. The initial filtering of data and quality control of data in ipyrad was completed as follows; filtering for adaptor sequences were done strictly (filter_adapters = 2), then only reads were retained with a strict minimum phred quality score (phred_Qscore_offset = 43). The first and last 5 bases of all reads were removed (max_low_qual_bases = 5), then reads with less than 50 bp discarded (filter_min_trim_len = 50). For de novo assembly, both minimum depth for statistical and minimum depth for majority-rule base calling was set to default (mindepth_statistical and mindepth_majrule = 6). The maximum cluster depth within samples was set to 10,000 (maxdepth = 10,000), clustering threshold for de novo assembly remained at default (clust_threshold = 0.85), the maximum number of alleles per site in consensus sequences was remained at default (max_alleles_consens = 2), maximum number of uncalled bases (max_alleles_consens) in consensus sequences and maximum heterozygotes in consensus sequences (max_Hs_consens) were both left to default at 0.05. The minimum number of samples per locus was set to 35 (min_samples_locus = 35), so each SNP would be present across a minimum of 35 samples, which corresponded to 50% minimum samples per locus (one sample failed to meet quality threshold for assembly and was discarded) to ensure effective population genotyping with 50% missing data or less (Shafer et al. 2016). The maximum number of SNPs per locus (max_SNPs_locus = 0.2), maximum number of indels per locus (max_Indels_locus = 5) was to set 5 and finally the maximum number heterozygous sites per locus (max_shared_Hs_locus = 0.5) remained at default. Lastly, the first five bases of all loci were trimmed (trim_loci = 5).

Genetic diversity, distance, and multivariate analysis

Descriptive statistics for genomic data were calculated with GenAlEx version 6.51b2 (Peakall and Smouse 2012). Comparative estimates of genetic distance (Nei 1978), analysis of molecular variance (AMOVA), and calculation of pairwise fixation index (F_{st}) were performed in GenAlEx with 10,000 permutations. Multivariate analysis of genetic

Table 2 Descriptive Statistics of Genomic Variation across native palms of the Leeward Antilles

Species	Population	PL* (%)	Na (SE)	PA	Ho (SE)	He (SE)	Fis
Sabal	Belnem	1.45%	0.945 (0.003)	0.009	0.014 (0.001)	0.007 (0.001)	-1.000
lougheediana	Lima	21.22%	1.211 (0.005)	0.153	0.019 (0.001)	0.026 (0.001)	0.077
	Montgomery	10.30%	1.016 (0.003)	0.073	0.028 (0.001)	0.027 (0.001)	-0.065
Sabal	Lagun	14.29%	1.091 (0.003)	0.030	0.033 (0.002)	0.058 (0.002)	0.353
antillensis	Seru Bientu	38.44%	1.386 (0.006)	0.103	0.059 (0.001)	0.100 (0.002)	0.303
	Seru Boosman	22.60%	1.220 (0.005)	0.026	0.070 (0.002)	0.081 (0.002)	0.075
	Seru Gracia	41.15%	1.413 (0.006)	0.111	0.056 (0.001)	0.110 (0.002)	0.361
	Viewpoint	34.88%	1.349 (0.006)	0.076	0.039 (0.001)	0.104 (0.002)	0.466
	Montgomery	23.12%	1.219 (0.005)	0.056	0.083 (0.002)	0.086 (0.002)	-0.015

*PL = polymorphic loci; Na = number of alleles per locus; PA = mean number of private alleles per population; Ho = observed heterozygosity; He = expected heterozygosity Fis = inbreeding coefficient.

distance (Orloci 1978; Huff et al. 1993) were performed in GenAlEx version 6.51b2 (Peakall and Smouse 2012). This PCA-based visualization method follows recent studies in population diversity and species delimitation using genomic data (Sunde et al. 2020, Piñeros et al. 2022), and resolves results similarly to other visualization methods (Azizi et al. 2017). An advantage of this multivariate analysis is that it does not require assignment of samples to a priori groups (such as required for DAPC), and thereby can avoid potential bias (Miller et al. 2020; Thia 2023), especially for species delimitation.

Population genetic structure

STRUCTURE v.2.3.4 (Pritchard et al., 2000) was used to determine the genetic structure and identify the most likely number of distinct genetic groups in the two *Sabal* species. STRUCTURE used a Bayesian algorithm to cluster samples into K distinct genetic groups by minimizing deviations from Hardy–Weinberg and linkage equilibrium within each cluster for K = 1–5 using 1,000,000 Markov chain Monte Carlo (MCMC) iterations after a burnin of 100,000 steps. Each analysis was repeated 60 times for each consecutive value of K. Results from STRUCTURE were then visualized using StructureSelector (Li and Liu 2018).

Assay of ex situ conservation effectiveness

We evaluated genetic distance and F_{st} values between ex situ collections and their source populations using GenAlEx. To estimate in situ alleles represented in ex situ collections, we calculated in situ private alleles as compared to ex situ collection for each species in GenAlEx, following methods established in Namoff et al. (2010) and Griffith et al. (2015, 2020). This provides an assay of how effective ex situ collections capture and steward diversity present in wild populations (Hoban et al. 2020).

Results

Sequencing and de-novo assembly

Illumina sequencing resulted in a total of 141,632,568 raw single end reads that ranged from 651,045 to 3,056,161 (mean = 65,1045) base pairs per sample. Initial quality control and filtering in ipyrad resulted in a total of 140,968,975 reads passing the filters that ranged from 647,621 to 3,041,828 (mean = 1,985,478.5) filtered reads. De-novo assembly of the reads using ipyrad generated 951,254 total clusters which ranged from 9,054 to 32,752 (mean cluster depth = 13,397.94) sequence clusters per sample, with 7,092 to 22,443 (average cluster depth = 9,707.3) being high depth clusters (defined as containing six or more reads for a minimum clustering depth). Considering only SNPs that were present in at least 50% of all individuals (minimum samples per locus), the final output from ipyrad resulted in 6,634 SNPs (uploaded to NCBI GenBank BioProject ID: PRJNA1073954 see Appendix 1).

Genetic diversity, distance, and multivariate analysis

The mean number of alleles per SNP ranged from 0.945 (Belnem, Bonaire) to 1.413 (Seru Gracia, Curaçao) (Table 2). The Lima population, i.e., the main group on Bonaire, had the greatest number of private alleles (0.153). Observed heterozygosity (H_o) ranged from H_o=0.014 for *S. lougheediana* in Belnem to H_o=0.083 for *S. antillensis* at Montgomery (ex situ), with a mean of H_o=0.042. Inbreeding coefficient (F_{is}) varied from F_{is} = -1.0 and indicated complete outbreeding in a single plant located at Belnem, Bonaire to F_{is} = 0.62 in the Viewpoint, Curaçao population, showing high levels of inbreeding. Comparing only the in situ plants between the two species (i.e. between the two islands) AMOVA found that the greatest genomic variation was within populations (76%), with 24% variation between

Table 3 Nei's Genetic Distance and F_{st} among populations. Nei's Genetic Distance values above diagonal, $\boldsymbol{F_{st}}$ values below diagonal

	Bonaire*	Bonaire Ex situ	Lagun	Seru Bientu	Seru Gracia	Seru Boosman	Viewpoint	Curaçao Ex situ	
Bonaire*	~	0.018	0.081	0.064	0.066	0.064	0.069	0.084	Bonaire*
Bonaire Ex situ	0.178	~	0.093	0.077	0.080	0.078	0.081	0.099	Bonaire Ex situ
Lagun	0.417	0.427	~	0.042	0.044	0.056	0.050	0.063	Lagun
Seru Bientu	0.315	0.301	0.150	~	0.018	0.034	0.026	0.028	Seru Bientu
Seru Gracia	0.313	0.299	0.146	0.063	~	0.033	0.016	0.040	Seru Gracia
Seru Boosman	0.369	0.347	0.209	0.109	0.101	~	0.040	0.058	Seru Boosman
Viewpoint	0.336	0.324	0.173	0.099	0.050	0.134	~	0.050	Viewpoint
Curaçao Ex situ	0.419	0.359	0.223	0.077	0.114	0.174	0.161	~	Curaçao Ex situ
	Bonaire*	Bonaire Ex situ	Lagun	Seru Bientu	Seru Gracia	Seru Boosman	Viewpoint	Curaçao Ex situ	

* The single specimen from Belnem (Table 1) is included with specimens from Lima to form a single "Bonaire" population for these statistics

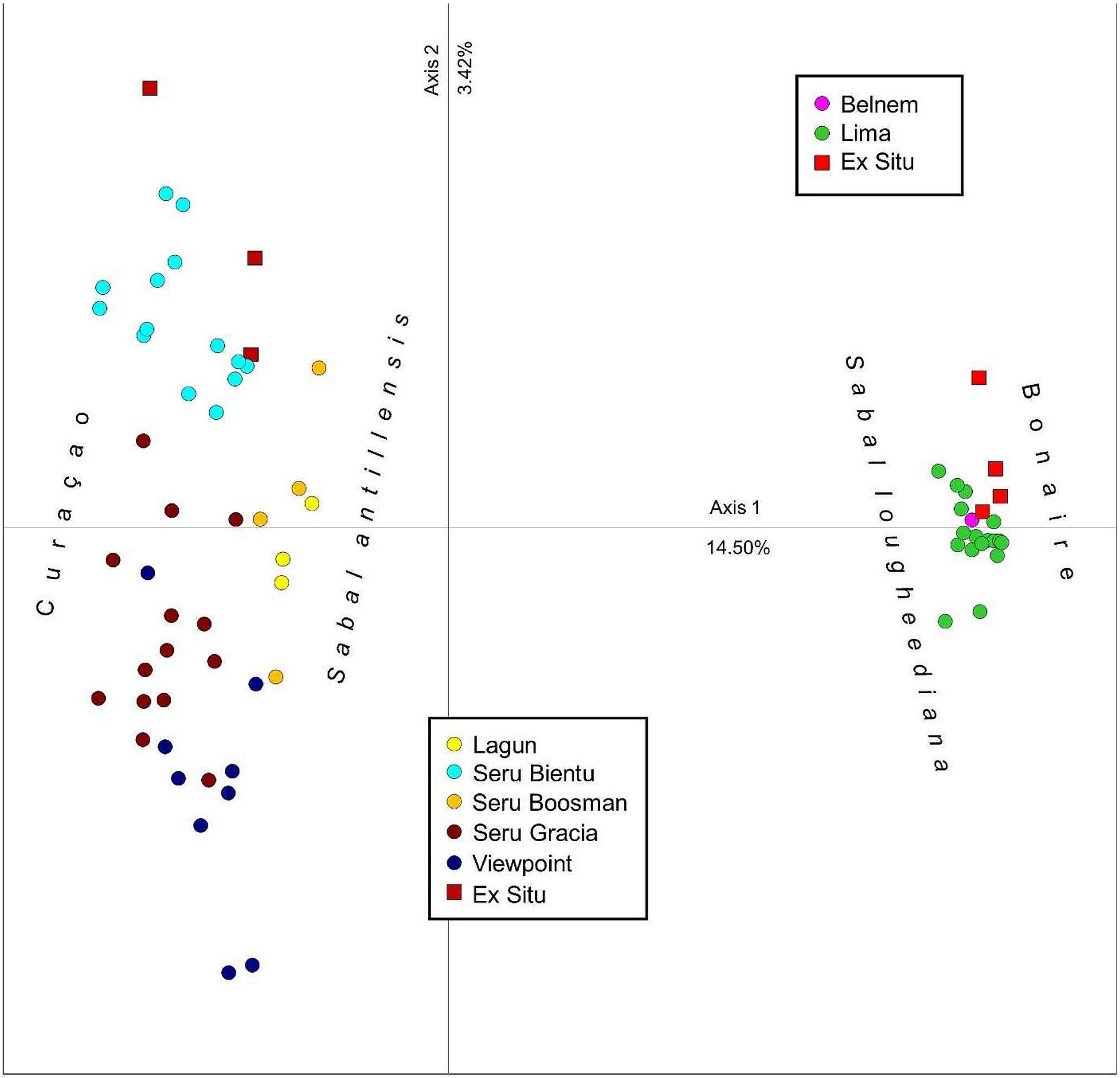

Fig. 4 Multivariate analysis of genetic distance data for native palms of the Leeward Antilles. Circles are in situ plants, squares are ex situ plants

the two species. Considering only in situ plants, the *Fst* between islands (i.e., between species) was 0.243. Parsed by population, *Fst* values ranged from 0.050 (between Seru Gracia and Viewpoint, two proximal sites on Curaçao; Fig. 3) to 0.370 (between Seru Boosman on Curaçao and the entire population on Bonaire) (Table 3). Nei's Genetic Distance values (Table 3) were closest between Seru Gracia and Viewpoint (0.016) and furthest between Bonaire and the population above Lagun (0.122). Multivariate analysis of genetic distance data is shown in Fig. 4. The first two axes account for 18% of the variation in the dataset. The first axis separates the two species into two distinct clusters that correspond to island groups, regardless of in situ or ex situ source. Populations from different hilltop locations in Curaçao are resolvable along the second axis.

Population structure

The results from STRUCTURE show that both the ΔK statistic and parsimony index suggested that the most likely number of genetic groups was K = 2 (ΔK = 8714.3764 (Fig. 5). In situ populations from Curaçao and Bonaire formed separate genetic clusters with some admixture in Curaçao, indicating gene flow from Bonaire to Curaçao (Fig. 5). Ex situ

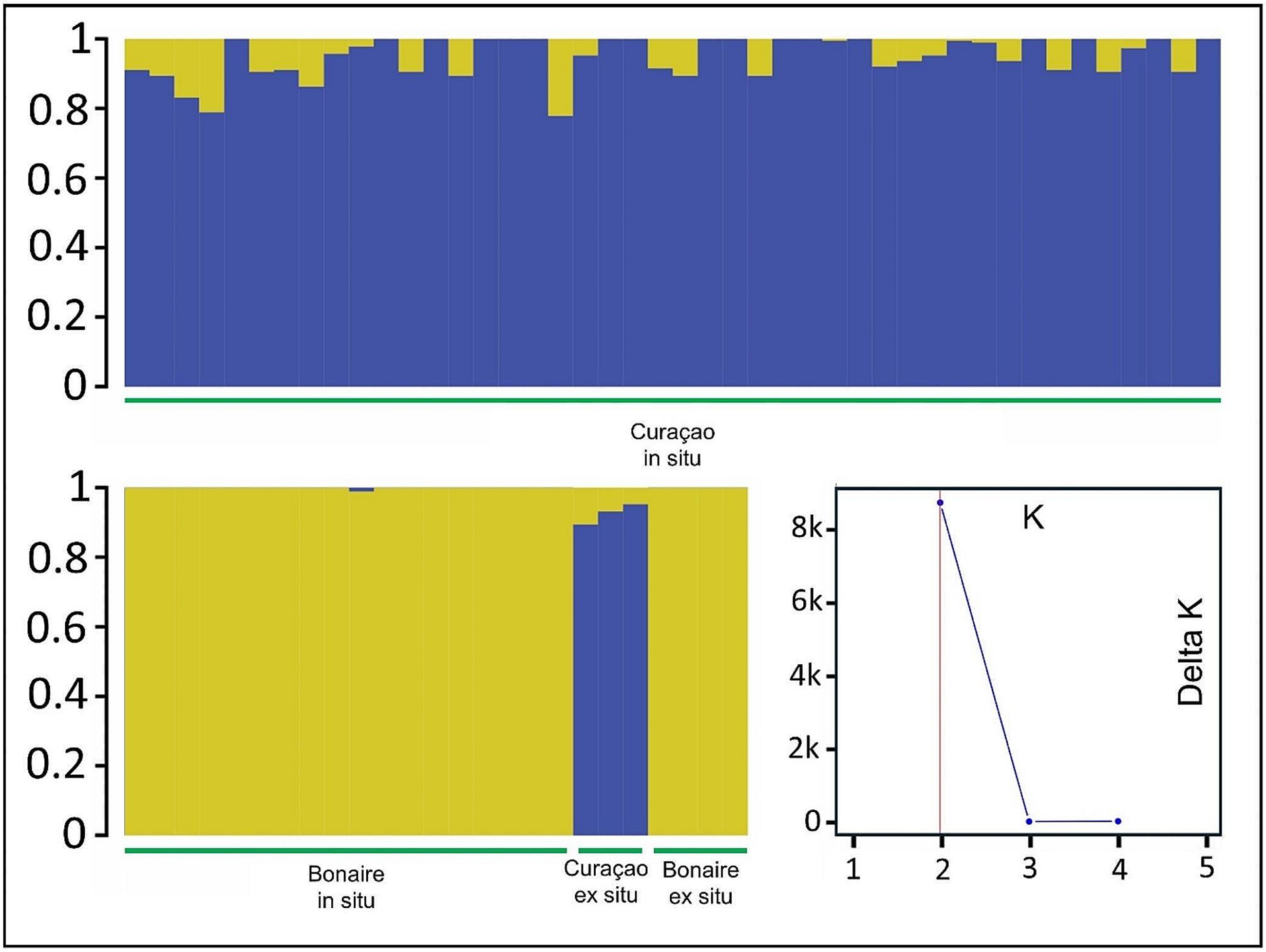

Fig. 5 Structure (Pritchard et al. 2000) histogram of genetic cluster membership generated from analysis of SNPs for K = 2, at which ΔK = 8,714.3764 (plot at lower left)

samples also assorted based on island source, with admixture in Curaçao and no evidence of admixture in Bonaire, indicating evidence of gene flow from Bonaire to Curaçao. Overall, these results show a similar pattern found in the multivariate analysis (Fig. 4).

Assay of ex situ conservation effectiveness

F_{st} and Nei's Genetic Distance statistics show closest affinity of the ex situ collections to their source populations. The Bonaire ex situ collection's least distance (0.054) and lowest F_{st} (0.178) is to the Bonaire population (Table 3). The Curaçao ex situ collection is closest to Seru Bientu (0.028 NGD / 0.077 F_{st}; Table 3). The 4 ex situ plants (= 4 maternal lines) from Bonaire capture 81% of all alleles from the island. The 3 ex situ plants (= 3 maternal lines) from Curaçao capture only 69% of alleles from the in situ plants on Curaçao, but capture 82% of the alleles from Seru Bientu, the specific source population for the ex situ seeds.

Discussion

The results presented above show both a very significant genomic separation between the two native palm species of the Leeward Antilles, and a close affinity of ex situ collections with their source populations. With the above data in place, we can now discuss genetic diversity of these species and address the two questions asked in the introduction – are these distinct species, and are they well represented in ex situ collections?

Genetic diversity and inbreeding

The genetic diversity measure for both species indicated that both *S. lougheediana* (H_o =0.014 to 0.028) and *S. antillensis* (H_o =0.033 to 0.083) show low levels of genetic diversity. It is possible that the low diversity is a consequence of isolation. Placing these results in a context highlights the imperilment of both taxa. For example, expansive

Butia eriospatha (Martius ex Drude) Beccari populations in Southern Brazil showed much higher H_o measures (between 0.21 and 0.55), yet were still considered at conservation risk due to limited recruitment (Nazareno and dos Reis 2014). Another Brazilian palm, *Acrocomia emensis* (Toledo) Lorenzi showed H_o values between 0.13 and 0.92, and was being targeted for conservation action due to limited genetic diversity (Neiva et al. 2016). Finally, *Copernicia prunifera* (Miller) HE Moore showed H_o values ranging from 0.37 to 0.39 across 14 populations in Northeastern Brazil (Costa et al. 2022). Given that species' high economic value, it was recommended to conserve these existing populations, and set aside habitat reserves to steward greater genetic diversity. By comparison, these examples serve to illustrate the relatively very reduced genetic diversity of the native Leeward Antillean palms, and highlight the need for conserving these native species.

In *S. antillensis* there is a difference between the measures of heterozygosity expected and observed, which indicates inbreeding may be playing a part in the population dynamics. For *S. lougheediana* although the single individual at Belnem shows complete outbreeding, the inbreeding coefficient (F_{IS}) indicates the populations fall closely to being within Hardy-Weinberg equilibrium, whereas populations of *S. antillensis* show a higher degree of inbreeding (F_{IS} = -0.015 to 0.466). This inbreeding, combined with such low level of allelic diversity indicates a potential bottleneck. This indicated bottleneck is consistent with the known history of the *S. antillensis* census, which shows a large recovery from a greatly reduced population over the last 40 years (Winkelman 1979; De Freitas et al. 2019). While populations of *S. antillensis* from Curaçao show minimal admixture with *S. lougheediana*, the low allelic diversity ($\leq$0.028) indicates that any potential gene flow is likely ancestral. While *Sabal* flowers are well established as entemophilous (Koptur et al. 2020), consistent and strong east-northeast prevailing winds (Chadee and Clarke 2014) could have potentially vectored pollen or pollen-laden insects from Bonaire to Curaçao. With the current genomic evidence suggesting historically larger past palm populations, both the pollen source and destination may have been more amenable to this gene flow than the current situation. It is also possible that the structure results reflect incomplete lineage sorting, as the two species may be closely related. While that hypothesis is outside the scope of the current study, it remains an interesting hypothesis for future testing.

Distinct species endemic to each island

Since these two prominent, highly-visible species evaded formal description until so recently (Griffith et al. 2017, 2019a) discussion with stakeholders prompts questions about synonomy: are these two species genetically distinct? Genomic data presented demonstrate a significant separation between these island taxa, with an F_{st} of 0.243. This level of fixation is greater than F_{st} values observed between many conspecific palm groups. For example, comparison among different date palm (*Phoenix dactylifera* L.) cultivars show F_{st} values between 0.01 and 0.16 (Gros-Balthazard et al. 2017; Saboori et al. 2021), The F_{st} value (0.243) reported here for the Leeward Antillean palms is more in line with published comparisons among palm species. For example, *Mauritiella* Burret species show an F_{st} values of 0.23 between species (Torres Jiménez et al. 2021), and *Phoenix* L. species show F_{st} values between 0.29 and 0.33 (Gros-Balthazard et al. 2017). Within the genus *Sabal*, observed F_{st} between *S. antillensis* and *S. lougheediana* exceeds values seen between *S. palmetto* (Walter) Lodd. ex Schult. & Schult.f., and *S. etonia* Swingle (F_{st} = 0.195) or between *S. palmetto* and *S. miamiensis* Zona (F_{st} = 0.161) (Grinage et al. in prep.). Fixation values therefore support the two taxa as distinct species. This distinct separation between *S. lougheediana* and *S. antillensis* on each respective island is also resolvable in the multivariate analysis in Fig. 4 and the Bayesian clustering in Fig. 5. In Fig. 4, the first axis clearly and significantly separates the two species, and the second axis separates intraspecific diversity. We infer that genetic diversity is higher for Curaçao's *S. antillensis* than Bonaire's *S. lougheediana* due to the much higher populations sizes on Curaçao and the observed recent bottleneck for *S. lougheediana*.

We concur with Lesica and Lavin (2023) that molecular data used to inform taxonomy must utilize robust sampling, or otherwise risk destabilizing nomenclature. While *Sabal* species have seen a number of molecular taxonomic studies (Ramp 1989; Goldman et al. 2011; Heyduk et al. 2016), the current work is the first to explore the *Sabal* of the Leeward Antilles, employs a thorough sampling (Nazareno et al. 2017), and employs more data than previous work. Results presented here therefore robustly confirm the hypothesis that *Sabal antillensis* and *Sabal lougheediana* are genomically distinct, contributing to nomenclatural stability. Such stability is a worthy goal on its own, but also ultimately fosters more effective conservation (Ely et al. 2017).

Ex situ conservation effectiveness

Parallel assays of ex situ collections alongside in situ population diversity studies can help guide and inform actions that can increase conservation effectiveness (Griffith et al. 2019b). The data presented here can help answer the question: are ex situ conservation efforts adequately representing the genetic diversity of each species? Genetic distance and fixation measures (Table 3) show how the ex situ collections share the highest affinity with their respective source populations, giving a first level of confidence in the results. This is also resolvable in the multivariate analysis (Fig. 4) and clustering analysis (Fig. 5). Assay of private alleles shows that these collections capture 81% (Bonaire) or 69% (Curaçao) of the observed in situ alleles. Compared to a widely-used global ex situ plant conservation target of 70% genetic diversity conserved per species (Convention on Biological Diversity 2020; Griffith et al. 2021a), the Bonaire collection meets the goal and the Curaçao collection just barely fails to achieve the goal. However, when compared to just its source population (Seru Bientu), the Curaçao ex situ collection captures 82% of in situ alleles. The tight relationship between the Curaçao collection and the hilltop population it was collected from are also reflected in genetic distance and fixation statistics (Table 3), as well as multivariate analysis (Fig. 4).

Conservation implications and recommendations

Significant conservation actions were set into motion following the description and especially the redlisting of *Sabal lougheediana* as Critically Endangered (Griffith and Coolen 2021). The results presented here, by confirming the uniqueness of both species, further support and justify those actions, as well as maintenance and expansion of herbivore management and exclusion on both islands (De Freitas et al. 2019; Engel et al. 2022). Plans to develop a Sabalpalm Park to encompass the entire current range of *S. lougheediana* on Bonaire will complement the protection offered by Christoffelpark which protects *S. antillensis* on Curaçao. The results also confirm that existing documented ex situ collections at Montgomery Botanical Center (22 maternal lines comprising 127 plants of *Sabal antillensis*, and 13 maternal lines comprising 149 plants of *Sabal lougheediana*) form the core of a genetic reserve in the case of unanticipated losses on either site, but also indicate that further development of such collections and metacollections is necessary. Such ex situ reserves should be grown at a number of sites to distribute risk of loss (Griffith et al. 2019b), as these taxa are at very few ex situ sites currently (Griffith et al. 2021b). Targeted efforts to augment the Curaçao ex situ collection with seeds collected from the other hilltop sites in Christoffelpark are recommended, in order to fully represent the breadth of genetic diversity throughout the species (Griffith et al. 2015). Efforts by nurseries on both islands to greatly increase availability of these native species for local horticulture is a critical action to augment and protect these species, both by serving as a directly accessible backup population as well as providing a marketable alternative to the import of nonnative *Sabal*. Making both species available in the international horticulture trade will also help reduce the threat of poaching (Kay et al. 2011), as evidence for poaching has been observed in the *Sabal lougheediana* population. Related to the observed gene flow from Bonaire to Curaçao, we also strongly recommend against planting nonnative *Sabal* species in landscapes on these islands. Besides the risk of inadvertent admixture (Klonner et al. 2017), the high risk of invasion of nonnative *Sabal* and potential introduction of pests and pathogens are major concerns (Van Buurt and Debrot 2012). The authors have observed nonnative *Sabal palmetto* seedlings germinating proximally to imported landscape palms at the Curaçao airport, demonstrating a clear potential for invasion. Following that observation, finally, we envision and encourage each island community to broadly and robustly adopt each of these two native palms, respectively, as a mainstay for civic, public, and private landscapes – a vivid, living symbol for native culture and conservation.

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Declarations

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